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Role of the Liver in Regulation of Ketone Body Production during Sepsis

ROBERT W. WANNEMACHER, JR., JUDITH G. PACE, FRANCIS A. BEALL, RICHARD E.

DINTERMAN, VANCE J. PETRELLA,\* and HAROLD A. NEUFELD

U. S. Army Medical Research Institute of Infectious Diseases,

Fort Detrick, Frederick, Maryland, 21701

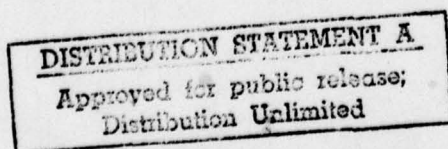
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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

Send proofs and reprint requests to: Dr. Robert W. Wannemacher, Jr., Ph.D.  
Senior Biochemist - USAMRIID  
Physical Sciences Division  
U.S. Army Medical Research Institute  
of Infectious Diseases  
Fort Detrick, Frederick, MD 21701

\* Current address: Gillette Medical Evaluation Laboratory  
1413 Research Boulevard  
Rockville, Maryland 20850



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20. (Continued) Some of the studies were repeated using tularemia as the model infection. From these studies, it was concluded that during pneumococcal sepsis, the failure of rats to become ketonemic during caloric deprivation was the result of reduced ketogenic capacity of the liver and a possibly decreased hepatic supply of fatty acids. The latter appeared to be a secondary consequence of a severe reduction in circulating plasma albumin, the major transport protein for fatty acids, with no effect on the degree of saturation of the albumin with free fatty acids. Also, the infection had no significant effect on the rate of lipolysis or release of fatty acids from adipose tissue. Ketone body utilization (oxidation) was either unaffected or reduced during pneumococcal sepsis in rats. Thus, a reduced rate of ketone production in the infected host was primarily responsible for the failure to develop starvation ketonemia under these conditions. The liver of the infected rat host shuttled the fatty acids away from  $\beta$ -oxidation and ketogenesis and toward triglyceride production, with resulting hepatocellular fatty metamorphosis.



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ABSTRACT During caloric deprivation, the septic host may fail to develop ketonemia as an adaptation to starvation. Since the plasma ketone body concentration is a function of the ratio of hepatic production and peripheral utilization, a pneumococcal sepsis model was utilized in rats to measure the complex metabolic events that could account for this failure, including the effects of infection on lipolysis and esterification in adipose tissue, fatty acid transport in plasma and the rates of hepatic ketogenesis and whole body oxidation of ketones. Some of the studies were repeated using tularemia as the model infection. From these studies, it was concluded that during pneumococcal sepsis, the failure of rats to become ketonemic during caloric deprivation was the result of reduced ketogenic capacity of the liver and a possibly decreased hepatic supply of fatty acids. The latter appeared to be a secondary consequence of a severe reduction in circulating plasma albumin, the major transport protein for fatty acids, with no effect on the degree of saturation of the albumin with free fatty acids. Also, the infection had no significant effect on the rate of lipolysis or release of fatty acids from adipose tissue. Ketone body utilization (oxidation) was either unaffected or reduced during pneumococcal sepsis in rats. Thus, a reduced rate of ketone production in the infected host was primarily responsible for the failure to develop starvation ketonemia under these conditions. The liver of the infected rat host shuttled the fatty acids away from  $\beta$ -oxidation and ketogenesis and toward triglyceride production, with resulting hepatocellular fatty metamorphosis.

### INTRODUCTION

During acute or prolonged starvation, the body increases its circulating plasma concentrations of ketones, and these are utilized as a source of energy by tissues such as skeletal muscle and eventually brain (1). This ketonemic adaptation to starvation reduces the need for energy derived from glucose calories, which in turn spares body protein by decreasing the rate of amino acid utilization for glucose synthesis. However, there is a general failure of ketonemic adaptation to occur during caloric deprivation associated with severe sepsis in man, monkey or rodent (2-6). Because of the lack of alternative fuels during severe sepsis, skeletal muscle breaks down protein and increases the utilization of amino acids, especially the branched-chain ones, as a source of energy (7). This results in an increased production and release from skeletal muscle of alanine and glutamine, the major substrates utilized by liver and kidney for gluconeogenesis, ureagenesis and ammoniogenesis. Thus, the failure of the severely septic host to develop starvation ketonemia can explain, in part, the marked increases in urea and ammonia nitrogen excretion and elevated rates of  $O_2$  consumption associated with the catabolic aspect of this disease (7, 8).

The rate of ketone body formation is a function of both the influx of free fatty acids from peripheral fat deposits to the liver and the extent of hepatic ketogenesis (9-13). The blood concentration of ketone bodies (acetoacetate and  $\beta$ -hydroxybutyrate) is a function of both the rate of hepatic production and peripheral utilization. During experimental starvation or alloxan diabetes in rats, the increase in

blood ketone concentration is due to a slight imbalance between the rates of production and utilization of the ketone bodies (14, 15).

Mechanisms responsible for a failure of starvation ketonemia to develop in the septic host have not been completely determined. Plasma free fatty acid concentrations have been reported to be variably increased or decreased during a number of gram-negative or gram-positive bacterial infections and viral illnesses in man and experimental animals (8, 16). When rats were made septic by cecal ligation and subjected to a 2-day fast period, plasma free fatty acid concentrations were decreased and fat mobilization appeared to be reduced as compared to measurements made in fasted control rats (5). In contrast, preliminary studies in perfused livers of infected rats showed a decrease in ketone body production from oleic acid but not from octanoic acid when compared to fasted controls (17). The present in vivo and in vitro studies were initiated to help elucidate the mechanisms by which sepsis is able to prevent the ketonemic adaptation to starvation during caloric deprivation in rats. A rat-virulent gram-positive coccus, Streptococcus pneumoniae, was employed to study the effects of sepsis on lypolysis and esterification in adipose tissue, fatty acid transport, hepatic ketogenesis and the rate of oxidation of ketone bodies. Some of the studies were repeated during infection with an obligate intracellular bacterium, Francisella tularensis.

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#### MATERIALS AND METHODS

Animals. Male, Fisher-Dunning rats, F-344/Mai J, (Microbiological Associates, Walkersville, Md.) weighing 175-200 g were utilized in these studies. Rats were maintained on a commercial diet (Wayne Lab Blox, Allied Mills, Inc., Chicago, Ill.) and water ad libitum for 7-10 days prior to experimental use. The environmental temperature was maintained at 76-78°F and a 12-h light-dark cycle was employed. At 0800 hours on the first day of each study, food was removed from the rats, and they were then injected subcutaneously (s.c.) with  $10^4$  virulent or heat-killed S. pneumoniae, Type 1, strain A5. Other rats were injected intraperitoneally (i.p.) with  $10^6$  live or heat-killed vaccine strain (LVS) F. tularensis organisms. Rats receiving heat-killed organisms served as controls for each study. Details concerning preparation of the bacterial inocula and the clinical manifestation of the infections have been published elsewhere (18,19). When appropriate, comparisons were also made in normal, nonfasted control rats.

In vitro rates of lypolysis and esterification in epididymal fat pads. Epididymal fat pads were obtained from fed or from fasted rats 24 h or 48 h after inoculation with either heat-killed or virulent S. pneumoniae. The fat pads were quickly removed, weighed and utilized to measure in vitro rates of production and release of glycerol and free fatty acids (FFA), as well as FFA esterification, by the procedure of Vaughan (20). One fat pad from each rat was used to determine initial concentration of FFA and glycerol, while the contralateral fat pad was incubated at 37°C for 1 h in Krebs' bicarbonate media which contained 3% fatty-acid-poor bovine albumin



(Calbiochem, San Diego, Calif.). At the end of the incubation period concentration of glycerol (21) and of FFA (22) in both tissue and media were determined by automated techniques.

In vivo rates of oxidation of pre-labeled body fat. Rats were injected intragastrically with 1 ml/100 g body wt of a solution that contained 6  $\mu$ Ci/ml of carboxyl- $^{14}$ C]tripalmitin (New England Nuclear, Boston, Mass.). Two hours later, each rat was injected intragastrically with 1 ml/100 g body wt of a 33% dextrose solution. For the next 7 days, rats were maintained on a 18% casein diet which supplied 40% of the calories from fat (23). At the end of 7 days, 6 rats were killed by cervical dislocation, immediately frozen in liquid nitrogen and the whole rat was pulverized in a precooled mortar and pestle. The lipid from 1 g of resulting powder was extracted by the procedure of McGarry and Foster (13) and analyzed for  $^{14}$ C] and for lipid content (18). Another sample of the resulting powder was dissolved in 2 N KOH and was analyzed for  $^{14}$ C] content.

Another group of 12 rats, which had been maintained for 7 days after their oral dose of labeled tripalmitin, were placed in individual, closed-circuit, metabolic cages that allowed for complete collection of urine, feces and expired  $\text{CO}_2$  (24). Air was drawn through the system at the rate of 0.7 liters/min, and  $\text{CO}_2$  was trapped in a mixture of IsoLab Scintisorb-C and Scintisol-GP, 8:1 (Isolab, Akron, Ohio). A sample (0.1 ml) of silicone antifoam compound (Sag 100, Union Carbide Corp., New York, N. Y.) was added per liter of scintillation mixture. A 5-ml aliquot was collected at 4, 8, 16, and 24 h and analyzed for  $^{14}$ C] activity (18) and  $\text{CO}_2$  content (24). The rats were allowed

access to food during the first day. On day 1 after being placed in the metabolic cages, food was removed; 6 rats were injected s.c. with virulent S. pneumoniae and 6 with heat-killed organisms. Complete expired CO<sub>2</sub>, urine, and feces collections were continued for the next 2 days and all samples were analyzed for [<sup>14</sup>C] content. Rats were then killed by cervical dislocation, and analyzed for lipid and [<sup>14</sup>C] content, as described before. These data were utilized to calculate the daily rate of [<sup>14</sup>CO<sub>2</sub>] production and percentage loss of radioactivity in total body lipid.

In vivo rates of fatty acid oxidation. At 24 or 48 h after rats were inoculated with either live or heat-killed S. pneumoniae, they were injected via the penile vein with either 1  $\mu$ Ci of 1-[<sup>14</sup>C]palmitic or 1-[<sup>14</sup>C]oleic acid (New England Nuclear) per 100 g body wt. Immediately thereafter rats were placed in individual, closed-circuit, metabolic cages (24). A 5-ml sample of the trapped CO<sub>2</sub> was removed at 0, 30, 60, 120, 180 and 240 min and analyzed for CO<sub>2</sub> and [<sup>14</sup>CO<sub>2</sub>] content as described previously (24). At the end of the 4 h, the rats were killed, blood was removed, and serum was analyzed for [<sup>14</sup>C] content. The procedures of Long et al. (25) were utilized to calculate the rates of fatty acid oxidation.

Measurement of plasma albumin and molar fatty acid/albumin ratio. Plasma was obtained from rats 24 and 48 h after inoculation of heat-killed or live S. pneumoniae. Plasma albumin concentrations were determined by automated immunoprecipitin techniques (26) employing rabbit anti-rat albumin and rat albumin standard (Cappell Laboratories, Cochranville, Pa.). Another sample of plasma was analyzed for FFA

content by automated procedures (22). The molar concentration of albumin was calculated by assuming that a gram of albumin was equivalent to 14.9  $\mu$ mol.

In vivo rate of disappearance of an i.v. lipid load. At 24 h after inoculation with either heat-killed or live *S. pneumoniae*, rats were injected via the penile vein with 20 mg of a lipid solution (Intralipid, Cutter Laboratories, Berkeley, Calif.) per 100 g body wt. Six infected and 6 control rats were killed at 0, 15, 30, 60, 120, 180 and 240 min after injection of lipid solution. Blood was collected in heparin and centrifuged; plasma was removed. Plasma was analyzed for triglycerides (21), fatty acids (22),  $\beta$ -hydroxybutyrate (27), cholesterol (21) and glucose (28) by automated procedures. Other infected and control rats were injected i.v. with 20 mg of lipid solution plus 1  $\mu$ Ci of [ $^{14}$ C]carboxyl-tripalmitin (New England Nuclear)/100 g body wt. Immediately following administration of the labeled lipid, rats were placed in individual, closed-circuit metabolic cages (24). A 5-ml aliquot of CO<sub>2</sub> absorbent was collected at 0, 15, 30, 60, 120, 180 and 240 min after injection of the labeled lipid and analyzed for [ $^{14}$ C] activity (18, 24).

Measurement of hepatic ketogenesis and fatty acid metabolism in vitro. Livers were removed from fed rats and those fasted 24-48 h after exposure to either live or heat-killed *S. pneumoniae* or *F. tularensis* (LVS). Liver perfusions were performed using a modification of the apparatus of Miller (29) as described previously (30). The perfusion medium consisted of 70 ml Krebs-Ringer bicarbonate buffer (pH 7.4), 30 ml of washed sheep erythrocytes, 3 g of fatty acid-poor,



bovine serum albumin (Calbiochem), and 500 units of heparin. The atmosphere in the closed perfusion system consisted of 95% of  $O_2$  and 5%  $CO_2$ . Perfusion of the liver was maintained at approximately 1 ml/min/g; the perfusate was recycled. At the end of a 60-min equilibration period, 2.5 ml of a 20 mM solution of either oleic or octanoic acid-albumin complex, which contained 1  $\mu$ Ci/ml of corresponding 1- $[^{14}C]$ oleic acid or 1- $[^{14}C]$ octanoic acid (New England Nuclear) was injected into the perfusion media. Following the initial pulse dose, labeled fatty acid solution was infused at the rate of 6 ml/h. The infusion rate was maintained constant by use of a syringe-driven constant infusion pump (Razel, Stamford, Conn.) and  $CO_2$  was collected in an absorbent-scintillation mixture (24, 31). A 3-ml sample of the perfusate was removed at 0, 15, 30, 45 and 60 min and analyzed for FFA (22),  $\beta$ -hydroxybutyrate (27) and acetoacetate (15). At the end of the 1-h infusion the liver was removed, frozen in liquid nitrogen, weighed and stored at  $-20^\circ C$ . A sample of the  $CO_2$  absorbent-scintillation fluid was analyzed for  $[^{14}C]$  content (18). A sample of the perfusate was saved for the determination of radioactivity in the ketone bodies by the procedure of McGarry and Foster (13, 31). The pulverized, frozen liver was analyzed for total lipid concentration and radioactivity (13, 31). The rate of ketogenesis was calculated from the slope of the plot of total ketones in the media vs. time.

Measurement of in vivo rates of  $[^{14}CO_2]$  production and oxidation of  $\beta$ -hydroxybutyrate and acetoacetate. At 24, 40 and 48 h after inoculation of rats with either live or heat-killed *S. pneumoniae*, the rats were injected via the penile vein with 1  $\mu$ Ci of  $\beta$ -3- $[^{14}C]$ hydroxybutyrate (New England Nuclear)/100 g body wt.



Immediately following the administration of the labeled ketone, rats were placed in individual closed-circuit metabolic cages (24). A 5-ml aliquot of the CO<sub>2</sub>-absorbent-scintillation mixture was collected at 0, 30, 60, 120, 180 and 240 min and analyzed for [<sup>14</sup>C] activity and CO<sub>2</sub> content. At the end of the experiment rats were removed from metabolic cages, anesthetized, and bled. Plasma was promptly separated and analyzed for  $\beta$ -hydroxybutyrate and acetoacetate concentrations (15, 27) and radioactivity (13, 31). In a similar study, rats were injected with 1  $\mu$ Ci of 3-[<sup>14</sup>C]acetoacetate (New England Nuclear)/100 g body wt at 24 h postinoculation of S. pneumoniae. The procedures of Long et al. (25) were utilized to calculate rates of labeled CO<sub>2</sub> production. Total body ketone concentrations were calculated by assuming a distribution space of 36% of body weight (14).

Statistical analysis. For sequential analysis within a group, data were analyzed by paired one-way analysis of variance. Intergroup comparisons were made by one-way analysis of variance. A (P) value of less than 0.01 was considered significant under the null hypothesis.

### RESULTS

By 24 h after inoculation of S. pneumoniae or LVS strain of F. tularensis, rats were characterized by an elevation of body temperature and other clinical indicators from the acute-phase of the infectious illness. In contrast, by 48 h after initiating each kind of infection, the rats had a decrease in body temperature, appeared morbid and died within the next 10 h. Therefore, they were considered to be in the agonal stage of the infection. Fasted rats given heat-killed organisms served as fasted controls for each infected group.

In vitro and in vivo effects of infection on rates of lipolysis and esterification. The weight of epididymal fat pads progressively decreased during fasting but no significant difference was observed between fat pads of rats inoculated with live or heat-killed S. pneumoniae (Table I). Both glycerol and FFA production was increased during the in vitro incubation of fat pads from fasted rats as compared to fed controls. However, no significant effect of S. pneumoniae infection was observed in these studies as compared to fasted controls (Table I). The calculated rate of fatty acid esterification was increased at 24 and 48 h in fasted rats and was slightly, but not significantly, slower in the fasted-infected group (Table I). The percentage of esterification of the fatty acids, produced as a result of lipolysis of triglycerides, was significantly less in fasted rats compared to fed controls and was not altered from fasting values in the presence of a pneumococcal infection.

When rats were given an oral dose of [ $^{14}\text{C}$ ]tripalmitin, followed in 1 h with a glucose load, approximately 85-90% of the label was associated with total body lipids 7 days later. When fed a purified diet (23), they lost 6.8% of the label per day in expired  $\text{CO}_2$  (Table II). During fasting, the rate of loss increased to 9.3 and 15% respectively at 24 and 48 h. Pneumococcal sepsis imposed upon fasting did not alter the rate of loss of label in the expired  $\text{CO}_2$  when compared to fasted controls.

Rates of fatty acid oxidation and transport of fatty acids in infected rats. When injected i.v. with labeled palmitic or oleic acid, the in vivo rates of [ $^{14}\text{CO}_2$ ] production were similar in fasted

controls and fasted pneumococci-infected rats (Table III). The rates of labeled  $\text{CO}_2$  production from oleic acid were significantly higher at 24 h than those observed when [ $^{14}\text{C}$ ]palmitic acid was utilized (Table III).

Both plasma albumin and FFA concentration were progressively decreased in the fasted pneumococci-infected rats as compared to fasted controls (Table IV). Maximum depression in plasma albumin and FFA concentrations were observed in the 48-h infected rats. The molar fatty acid/albumin ratio was approximately 2 in fasted controls and was not significantly different in the infected group at 24 and 48 h.

Triglyceride disposal and fatty acid metabolism in infected rat.

Following an i.v. lipid load, triglyceride accumulation and disappearance was significantly faster in fasted rats 24 h after inoculation with S. pneumoniae compared to fasted controls (Fig. 1). The accumulation of FFA in plasma was similar in both groups, but the rate of disappearance was significantly faster in infected rats than in fasted controls (Fig. 1). Despite the more rapid disappearance of plasma triglycerides and FFA, accumulation of plasma  $\beta$ -hydroxybutyrate and cholesterol was significantly less in the fasted-infected group than in fasted controls. No significant differences were noted in the rate of labeled  $\text{CO}_2$  production from [ $^{14}\text{C}$ ]triglycerides or in plasma glucose concentrations between fasted-infected and fasted-control rats.

Effect of infection on hepatic ketogenesis and fatty acid metabolism by the perfused liver. To determine the effects of infection on hepatic ketogenesis, isolated livers from fed controls



and fasted rats inoculated with either heat-killed (fasted controls) or live S. pneumoniae or LVS were perfused with either 20 mM oleic or octanoic acid. Data are presented in Table V and ketogenesis is expressed as  $\mu\text{mol}$  of ketones ( $\beta$ -hydroxybutyrate + acetoacetate) produced/min/100 g body wt. When the livers were perfused with the Krebs-Ringer bicarbonate plus 3% albumin and no added fatty acids, the rates of ketogenesis were very low and not significantly different between fed, fasted, or fasted-infected group (Table V). The addition of 20 mM oleic acid to the perfusate resulted in a doubling of the rate of ketogenesis in the livers of fed rats and a 4-fold increase in livers from rats fasted for 24 or 48 h. The rate of ketogenesis from oleic acid was significantly reduced in livers of fasted rats 48 h after inoculation of S. pneumoniae or LVS compared to fasted controls given the heat-killed organisms (Table V). When the livers were perfused with 20 mM octanoic acid, the rates of ketogenesis were slightly higher in the fed group and lower in the fasted groups compared to livers perfused with oleic acid. No significant difference was observed in the rate of ketogenesis from octanoic acid between livers from fasted controls or fasted-infected rats (Table V). Similar rates of uptake of oleic acid by perfused livers were observed in fasted rats 48 h after exposure to either live or heat-killed S. pneumoniae but this rate was significantly reduced in the livers from the rats 48 h after inoculation with F. tularensis (Table VI).

When [ $^{14}\text{C}$ ]oleic acid was added to the perfusate, the rate of hepatic utilization of the label and [ $^{14}\text{CO}_2$ ] production was similar in livers from fasted rats studied 48 h after inoculation by the



heat-killed or live S. pneumoniae. In contrast, incorporation of label into ketones was significantly reduced while the formation of labeled lipids was significantly increased in the livers of fasted-infected rats compared to the fasted controls (Table VII). Livers from fasted rats had a reduced weight and lipid content compared to those of the fed group (Table VIII). Livers from fasted-infected rats were increased in both weight and lipid content as compared to fed controls. When studied 60 min after the addition of either labeled octanoic or oleic acid, the liver lipids from both the fed and fasted-infected rats contained more radioactivity than did the liver lipids of fasted control rats given heat-killed organisms. Livers from the fasted rats 48 h after inoculation with S. pneumoniae incorporated significantly more of either labeled octanoic or oleic acid into the lipid fraction than livers from rats studied at 24 h (Table VIII). In all groups the rate of incorporation of labeled octanoic acid into total lipid was significantly less than when [ $^{14}\text{C}$ ]oleic acid was perfused.

In vivo rate of utilization of ketone by infected rats.

Following an i.v. injection of  $\beta$ -[ $^{14}\text{C}$ ]hydroxybutyrate, the rate of labeled  $\text{CO}_2$  production was similar in fasted rats inoculated 24 or 40 h previously with either heat-killed or live organisms. In contrast, the rate of [ $^{14}\text{CO}_2$ ] production was significantly decreased in fasted-infected rats 48 h after inoculation of S. pneumoniae in comparison to fasted controls (Table IX). Also, after the administration of [ $^{14}\text{C}$ ]acetoacetate, rates of labeled  $\text{CO}_2$  production were similar in all rats at 24 h (Table IX). Total body concentration of

$\beta$ -hydroxybutyrate or acetoacetate was progressively and significantly reduced in fasted-infected rats compared to fasted controls (Table IX). Because of this reduction in body ketone content, the rate of ketone oxidation was significantly reduced in fasted-infected rats, with the most significant reduction during the latter stages of pneumococcal infection (Table IX).

#### DISCUSSION

It has been documented in several laboratories (2-6) that sepsis, alone or in combination with trauma, prevents the ketonemia usually associated with caloric restriction. Since plasma ketone concentrations are the algebraic sum of the difference between the rate of production and utilization (14, 15), the failure to develop ketonemia in the septic host could be the result of decreased synthesis, increased rates of utilization, or a combination both responses. The major ketones of the body ( $\beta$ -hydroxybutyrate and acetoacetate) are synthesized principally in the liver and the synthetic process can be influenced by both FFA supply and hepatic regulation of the rate of ketosis (9-13).

The in vitro model of Vaughan (20) was utilized to measure rates of lipolysis and esterification in the epididymal fat pads of the rat during pneumococcal sepsis. In this model it is assumed that any glycerol produced during the in vitro lipolysis of fat pad triglycerides is neither metabolized to any extent nor re-utilized for triglyceride synthesis. As reported by Vaughan (20), fasting led to an increased glycerol production and a decrease in the

percentage of FFA which are esterified to triglycerides. When an S. pneumoniae infection was superimposed on fasting, it had no significant effect on the rates of either lipolysis or esterification in isolated epididymal fat pads. To help validate this conclusion, an in vivo model was developed in which total body fat was prelabeled with [ $^{14}\text{C}$ ] and the rates of labeled  $\text{CO}_2$  production were measured during fasting alone or with a superimposed S. pneumoniae infection. Fasting for 24 or 48 h significantly increased the rate of [ $^{14}\text{CO}_2$ ] production from labeled body lipids as compared to values obtained in fed rats, however; infection plus fasting did not significantly alter this effect.

Thus, from both in vivo and in vitro studies of depot fat, it may be concluded that the inhibition of calorie-restricted ketonemia during pneumococcal sepsis in rats is not the result of altered rates of lipolysis or esterification. In contrast, Ryan et al. (5) observed a decrease in normal, fasting-induced mobilization of fat during peritonitis induced by ligation of the cecum of the rat. It is possible, therefore, that caloric restriction during gram-negative sepsis which is accompanied by endotoxemia could result in decreased rates of lipolysis and elevated rates of fatty acid esterification in adipose tissue, which could then in part prevent the ketonemia associated with caloric restriction. However, the possibility of an endotoxin interaction did not exist in the gram-positive S. pneumoniae model utilized in these studies.

In general, plasma FFA concentrations are decreased during sepsis in man and experimental animals (5, 6, 8, 16). This decrease can be the result of an increased rate of utilization, a decrease in the



concentration of the FFA transport protein (albumin), and/or depression in the rates of release of fatty acids from adipose tissue. While plasma FFA concentrations were decreased during pneumococcal sepsis, no differences were observed in the rates of oxidation of labeled palmitic or oleic acid as compared to fasted controls. In contrast, plasma albumin concentrations were markedly reduced during pneumococcal sepsis in these rats, but the molar fatty acid/albumin ratio was very similar to that observed in fasted controls. Thus, decreased plasma FFA content was correlated with the depression in plasma albumin concentration in fasted-infected rats and was not related either to altered rates of fatty acid oxidation or to inhibited release from adipose tissue. It cannot be determined from these studies whether reduced plasma albumin concentrations had an effect on rate of uptake of FFA by the liver of infected rats. In contrast to these findings during infection, the depression in plasma FFA concentration during the feeding of a high carbohydrate diet or insulin treatment is a result of a marked reduction in a degree of saturation of plasma albumin with FFA because of the reduced lipolysis and release of FFA from adipose tissue. While both sepsis and hyperinsulinemia can result in marked reduction of plasma FFA content, the mechanisms are quite different and will require future studies to determine if both processes have similar effects on the rate of uptake of these acids by the liver. This possibility can only be tested by measurement of arterial-venous differences across the liver of the infected host in vitro or in vivo.

To overcome the possible effects of fatty acid supply, in vivo and in vitro studies on the effect of infection on hepatic rates of



ketogenesis were carried out with excess exogenous source of fatty acids. When an i.v. lipid load was given to a rat during pneumococcal sepsis, plasma triglyceride and FFA disappearance was faster than that observed in fasted controls. Despite an elevated rate of breakdown of triglycerides and utilization of FFA, a slower accumulation of plasma  $\beta$ -hydroxybutyrate and cholesterol was observed in the septic rat as compared to its fasted controls. These data suggest that during pneumococcal sepsis the liver was not utilizing FFA for ketone body production and cholesterol synthesis at the same rate as that seen in control rats. Since fatty acids must undergo  $\beta$ -oxidation to form the acetyl group which can then be utilized for ketone body or cholesterol synthesis (13, 31, 32), the data in this in vivo study suggest that pneumococcal sepsis has altered hepatic metabolism of FFA.

To evaluate further the effects of infection on rates of hepatic ketogenesis studies were carried out with isolated livers perfused with long- and medium-chain fatty acids. When oleic acid (a long-chain FFA) was utilized as a substrate to study regulation of ketogenesis, livers from fasted rats had a 3-fold enhancement in the rate of ketone production compared to those of fed rats. This effect of fasting is very similar to the observations reported by McGarry and Foster (31) and indicates that the procedure utilized in these studies was an adequate model for studying the regulation of ketogenesis in the isolated perfused liver system. The livers from fasted rats 48 h after inoculation of S. pneumoniae or 24 and 48 h after F. tularensis had much lower rates of ketogenesis compared to fasted noninfected

controls. These observations clearly indicated that both of these infections depressed the ketogenic capacity of the liver when the rat was subjected to complete caloric restriction. In livers from rats infected with pneumococci, the reduced rate of ketogenesis was not related to an altered rate of uptake of oleic acid by the perfused livers. A slight decrease in the rate of uptake of oleic acid was observed in livers from the rats 48 h after inoculation with the LVS strain of F. tularensis. At this stage of experimental tularemia in rats (33), hepatic damage can be detected by microscopic and biochemical analyses. Thus, the reduced uptake of oleic acid by perfused livers from rats in the later stages of the F. tularensis infection may be related to hepatic damage.

When octanoic acid (a medium-chain FFA) was utilized as a substrate to study regulation of ketogenesis in isolated perfused rat liver, only very minimal enhancement of ketone production was observed in livers of fasted rats compared to those in the fed group. Further, neither the S. pneumoniae nor F. tularensis infection had any effect on the rate of ketogenesis when isolated livers were perfused with this medium-chain fatty acid. Data from these studies support the conclusions of McGarry and Foster (13, 31, 34-36) that the major regulatory mechanisms for the oxidation of long-chain fatty acids is not operative when medium-chain fatty acids serve as a ketogenic substrate.

McGarry and coworkers (13, 31, 34-37) have presented evidence to indicate that the increased ketogenic capacity of livers from starved or diabetic rats appears to result mainly from an increased ability

of the liver to transport activated fatty acids from cytosol to the mitochondria. The transport of long-chain fatty acids into mitochondria is catalyzed by "carnitine acyltransferase" (carnitine palmitoyltransferase, EC 2.3.21) which is an enzyme system comprised of carnitine acyltransferase I and II (32, 38, 39). Transferase I is believed to catalyze conversion of long-chain fatty acyl-CoA and carnitine into acylcarnitine and CoA-SH and is believed to take place on the outer aspects of the inner mitochondrial membrane (38, 39). This is followed by the reverse reaction which utilizes an intramitochondrial CoA-SH pool and is catalyzed by transferase II on the inner aspect of the inner mitochondrial membrane (38, 39). More recently, McGarry and coworkers (40) have shown the correlation between hepatic carnitine concentration and the ketogenic capacity of this tissue, which suggests an important relationship between the carnitine transferase I system and the regulation of long-chain hepatic fatty acid oxidation and ketogenesis. These workers have also presented evidence which suggests that carnitine transferase II plays an equally important role in the regulation of ketogenesis (36). In contrast to the long-chain fatty acids, the medium-chain fatty acids, such as octanoic, do not require the carnitine acyltransferase enzyme system to enter the mitochondria (37). Since the infection-related decrease in ketogenic capacity of liver was only observed when long-chain fatty acids were utilized as substrate, it may be hypothesized that the infectious process exerts some inhibitory effects on a carnitine acyltransferase system of the liver.



Although the exact stimulus for the activation of hepatic ketogenic capacity of the liver has not been elucidated, McGarry, Wright and Foster (37) have shown that the *in vivo* injection of anti-insulin serum or glucagon rapidly increases the ketogenic capacity of the liver from a fed rat. This has led to the conclusion that hepatic ketogenesis is under bihormonal control with glucagon being stimulatory and insulin inhibitory (37). During pneumococcal sepsis in rats both portal vein and inferior vena cava concentrations of immunoreactive glucagon and insulin are increased very early in the infection with a significant reduction in the insulin/glucagon ratio (41). Similar increases in plasma glucagon and insulin and depression in insulin/glucagon molar ratio have been observed during bacterial and viral infections in man (42, 43) and monkey (44). From this profile of glucagon and insulin, the ketogenic capacity of liver would be expected to be increased in the infected host instead of decreased as observed in these studies. If, as has been suggested (37), hepatic fatty acid metabolism and ketogenesis are under bihormonal control of glucagon and insulin, then future studies are needed to determine why livers from infected hosts have reduced ketogenic capacity despite a hormonal profile which should stimulate hepatic ketone production.

Data on the [ $^{14}\text{CO}_2$ ] production from oleic acid, employing the calculations of McGarry and Foster (13, 31), was utilized to show that Krebs cycle activity was not significantly altered in the liver of fasted rats during pneumococcal sepsis. In contrast, the amount of labeled oleic acid contributing to the ketone pool was markedly decreased and that in the lipid fraction was markedly elevated in fasted-infected rats compared to fasted controls. This suggests that

a larger portion of the oleic acid was utilized for the synthesis of triglycerides and phospholipids in the liver of the infected rat. This conclusion was supported by the progressive increase in lipid content of livers from the fasted-infected rats compared to fasted controls. Fiser et al. (45) also reported an accelerated rate of utilization of FFA for triglyceride synthesis during endotoxic fever in monkeys. This increase in triglyceride synthesis could help to explain the fatty metamorphosis that has been observed in hepatocytes during sepsis (46-48).

Livers from fasted-infected rats also incorporated labeled carbon from octanoic acid into liver lipids at a faster rate than did their fasted controls. Since octanoate is not known to be utilized directly for triglyceride synthesis (13, 32, 36), an increased amount of the acetyl-CoA, from  $\beta$ -oxidation in the mitochondria, was transported to the cytoplasm and subsequently utilized for the synthesis of C-16 and C-18 fatty acids in livers from infected rats. An acceleration of lipogenesis in isolated hepatocytes of infected rats has also been suggested in the in vitro studies of Canonico and his colleagues (49). Recently, it has been reported that malonyl-CoA, an intermediate in the synthesis of fatty acids from acetyl-CoA, inhibits the carnitine acyltransferase I enzyme of liver and may play a role in regulation of the rate of hepatic ketogenesis in meal-fed rats (50, 51).

From the present studies, it may be concluded that the general failure of ketonemic adaptation to starvation to occur during the caloric deprivation associated with severe sepsis is a result of both a reduced ketogenic capacity of the liver and a possibly decreased supply of fatty acids. During pneumococcal sepsis in the rat, the

decrease in plasma fatty acids is accompanied by a reduction in circulating albumin, the major transport protein for fatty acids, with no effect on the degree of saturation of the albumin with fatty acids. Further, from the studies with labeled  $\beta$ -hydroxybutyrate and acetoacetate, it is apparent that the failure of ketonemia to develop during caloric restriction in the infected host is probably not the result of increased utilization of ketones by peripheral tissues. In fact, during the agonal stages of pneumococcal sepsis in the rat, ketone utilization was depressed when compared to fasted controls. Because of the marked decrease in the total body ketone pool, these substrates were utilized at a markedly reduced rate as a source of energy by the infected rat host. This resulted in a marked increase in the breakdown of body protein and utilization of amino acids as a source of energy with a resulting increase in gluconeogenesis, ureagenesis and ammoniogenesis (7, 8).



REFERENCES

1. Cahill, G. F. Jr., T. T. Aoki, and E. B. Marliss. 1971. Insulin and muscle protein. In Handbook of Physiology, Sect. 7: Endocrinology. American Physiological Society, Washington. 1: 563-577.
2. Flatt, J.-P., and G. L. Blackburn. 1974. The metabolic fuel regulatory system: implications for protein-sparing therapies during caloric deprivation and disease. Am. J. Clin. Nutr. 27: 175-187.
3. Border, J. R., R. Chenier, R. H. McManamy, J. LaDuca, R. Seibel, R. Birkhahn, and L. Yu. 1976. Multiple systems organ failure: muscle fuel deficit with visceral protein malnutrition. Surg. Clin. North Am. 56: 1147-1167.
4. Wannemacher, R. W., Jr., M. V. Kaminski, Jr., H. A. Neufeld, R. E. Dinterman, K. A. Bostian, and C. L. Hadick. 1978. Protein sparing therapy during pneumococcal sepsis in rhesus monkeys. J. Parenteral Enteral Nutr., in press.
5. Ryan, N. T., G. L. Blackburn, and G. H. A. Clowes, Jr. 1974. Differential tissue sensitivity to elevated endogenous insulin levels during experimental peritonitis in rats. Metabolism 23: 1031-1089.
6. Neufeld H. A., J. A. Pace, and F. E. White. 1976. The effect of bacterial infections on ketone concentrations in rat liver and blood and on free fatty acid concentrations in rat blood. Metabolism 25: 877-884.

7. Wannemacher, R. W., Jr. 1977. Key role of various individual amino acids in host response to infection. Am. J. Clin. Nutr. 30: 1269-1280.
8. Blackburn, G. L. 1977. Lipid metabolism in infection. Am. J. Clin. Nutr. 30: 1321-1332.
9. Seyffert, W. A., Jr., and L. L. Madison. 1967. Physiological effects of metabolic fuels on carbohydrate metabolism. I. Acute effect of elevation of plasma free fatty acids on hepatic glucose output, peripheral glucose utilization, serum insulin, and plasma glucagon levels. Diabetes. 16: 765-776.
10. Foster, D. W. 1967. Studies in the ketosis of fasting. J. Clin Invest. 46: 1283-1296.
11. Crespin, S. R., W. B. Greenough, III, and D. Steinberg. 1969. Stimulation of insulin secretion by infusion of free fatty acids. J. Clin. Invest. 48: 1934-1943.
12. Williamson, D. H., D. Veloso, E. V. Ellington, and H. A. Kerbs. 1969. Changes in the concentrations of hepatic metabolites on administration of dihydroxyacetone or glycerol to standard rats and their relationship to the control of ketogenesis. Biochem. J. 114: 575-584.
13. McGarry, J. D., and D. W. Foster. 1971. The regulation of ketogenesis from octanoic acid. The role of the tricarboxylic acid cycle and fatty acid synthesis. J. Biol. Chem. 246: 1149-1159.
14. Bates, M. W., H. A. Kerbs, and D. H. Williamson. 1968. Turnover rates of ketone bodies in normal, starved and alloxan-diabetic rats. Biochem. J. 110: 655-661.

15. McGarry, J. D., M. J. Guest, and D. W. Foster. 1970. Ketone body metabolism in the ketosis of starvation and alloxan diabetes. J. Biol. Chem. 245: 4382-4390.
16. Beisel, W. R., and R. H. Fiser, Jr. 1970. Lipid metabolism during infectious illness. Am. J. Clin. Nutr. 23: 1069-1079.
17. Pace, J. A., F. A. Beall, H. A. Neufeld, and R. W. Wannemacher Jr. 1977. Alterations in carnitine (Car) acylation states in S. pneumoniae infected (INF) rats. Fed. Proc. 36: 788 (abstr.).
18. Wannemacher, R. W., Jr., M. C. Powanda, R. S. Pekarek, and W. R. Beisel. 1971. Tissue amino acid flux after exposure of rats to Diplococcus pneumoniae. Infect. Immun. 4: 556-562.
19. Powanda, M. C., R. E. Dinterman, R. W. Wannemacher, Jr., and G. D. Herbrandson. 1974. Distribution and metabolism of phenylalanine and tyrosine during tularaemia in the rat. Biochem. J. 144: 173-176.
20. Vaughan, M. 1962. The production and release of glycerol by adipose tissue incubated in vitro. J. Biol. Chem. 237: 3354-3358.
21. Rush, R. L., L. Leon, and J. Turrell. 1970. Automated simultaneous cholesterol and triglyceride determination on the AutoAnalyzer II instrument. In Advances in Automated Analysis. Technicon International Congr. 1: 503-507.
22. Dalton, C., and C. Kowalski. 1967. Automated colorimetric determination of free fatty acids in biological fluids. Clin. Chem. 13: 744-751.
23. Allison, J. B., R. W. Wannemacher, Jr., W. L. Banks, Jr., and W. H. Wunner. 1964. The magnitude and significance of the protein reserves in rats fed at various levels of nitrogen. J. Nutr. 84: 383-388.



24. Wannemacher, R. W., Jr., A. S. Klainer, R. E. Dinterman, and W. R. Beisel. 1976. The significance and mechanism of an increased serum phenylalanine-tyrosine ratio during infection. Am. J. Clin. Nutr. 29: 997-1006.
25. Long, C. L., J. L. Spencer, J. M. Kinney, and J. W. Geiger. 1971. Carbohydrate metabolism in normal man and effect of glucose infusion. J. Appl. Physiol 31: 102-109.
26. Bostian, K. A., B. S. Blackburn, R. W. Wannemacher, Jr., V. G. McGann, W. R. Beisel, and H. L. Dupont. 1976. Sequential changes in the concentration of specific serum proteins during typhoid fever infection in man. J. Lab. Clin. Med. 87: 577-585.
27. Zivin, J. A., and J. F. Snarr. 1973. An automated colorimetric method for the measurement of 3-hydroxybutyrate concentration. Anal. Biochem. 52: 456-461.
28. Asrow, G. 1969. Semiautomated enzymic micro methods for blood glucose and lactic acid on a single filtrate. Anal. Biochem. 28: 130-138.
29. Miller, L. L. 1961. Some direct actions of insulin, glucagon, and hydrocortisone on the isolated perfused rat liver. Recent Progr. Hormone Res. 17: 539-568.
30. Zenser, T. V., F. R. DeRubertis, and R. T. Curnow. 1974. Effects of prostaglandins on hepatic adenylate cyclase activity and cyclic adenosine 3',5'-monophosphate content. Endocrinology 94: 1404-1410.
31. McGarry, J. D., and D. W. Foster. 1971. The regulation of ketogenesis from oleic acid and the influence of antiketogenic agents. J. Biol. Chem. 246: 6247-6253.

32. Fritz, I. B. 1961. Factors influencing the rates of long-chain fatty acid oxidation and synthesis in mammalian systems. Physiol. Rev. 41: 52-129.
33. Powanda, M. C., G. L. Cockerell, J. B. Moe, F. B. Abeles, R. S. Pekarek, and P. G. Canonico. 1975. Induced metabolic sequelae of tularemia in the rat: correlation with tissue damage. Am. J. Physiol. 229: 479-483.
34. McGarry, J. D., and D. W. Foster. 1972. Regulation of ketogenesis and clinical aspects of the ketotic state. Metabolism 21: 471-489.
35. McGarry, J. D., J. M. Meier, and D. W. Foster. 1973. The effects of starvation and refeeding on carbohydrate and lipid metabolism in vivo and in the perfused rat liver. The relationship between fatty acid oxidation and esterification in the regulation of ketogenesis. J. Biol. Chem. 248: 270-278.
36. McGarry, J. D., and D. W. Foster. 1974. The metabolism of (-)-octanoylcarnitine in perfused livers from fed and fasted rats. J. Biol. Chem. 249: 7984-7990.
37. McGarry, J. D., P. H. Wright, and D. W. Foster. 1975. Hormonal control of ketogenesis. Rapid activation of hepatic ketogenic capacity in fed rats by anti-insulin serum and glucagon. J. Clin. Invest. 55: 1202-1209.
38. Kopec, B., and I. B. Fritz. 1973. Comparison of properties of carnitine palmitoyltransferase I with those of carnitine palmitoyltransferase II, and preparation of antibodies to carnitine palmitoyltransferases. J. Biol. Chem. 248: 4069-4074.

39. Brosnan, J. T., B. Kopec, and I. B. Fritz. 1973. The localization of carnitine palmitoyltransferase on the inner membrane of bovine liver mitochondria. J. Biol. Chem. 248: 4075-4082.
40. McGarry, J. D., C. Robles-Valdes, and D. W. Foster. 1975. Role of carnitine in hepatic ketogenesis. Proc. Natl. Acad. Sci. U. S. A. 72: 4385-4388.
41. Zenzer, T. V., F. R. DeRubertis, D. T. George, and E. J. Rayfield. 1974. Infection-induced hyperglucagonemia and altered hepatic response to glucagon in the rat. Am. J. Physiol. 227: 1299-1305.
42. Rocha, D. M., F. Santeusano, G. R. Faloona, and R. H. Unger. 1973. Abnormal pancreatic alpha-cell function in bacterial infections. N. Engl. J. Med. 288: 700-703.
43. Rayfield, E. J., R. T. Curnow, D. T. George, and W. R. Beisel. 1973. Impaired carbohydrate metabolism during a mild viral illness. N. Engl. J. Med. 289: 618-621.
44. George, D. T., E. J. Rayfield, and R. W. Wannemacher, Jr. 1974. Altered glucoregulatory hormones during acute pneumococcal sepsis in the rhesus monkey. Diabetes 23: 544-549.
45. Fiser, R. H., J. C. Denniston, and W. R. Beisel. 1974. Endotoxemia in the rhesus monkey: alterations in host lipid and carbohydrate metabolism. Pediatr. Res. 8: 13-17.
46. von Brand T., and T. I. Mercado. 1958. Quantitative and histochemical studies on liver lipids of rats infected with Plasmodium berghei. Am. J. Trop. Med. Hyg. 67: 311-320.



47. Hirsch, R. L., D. G. MacKay, R. I. Travers, and R. K. Skraly.  
1964. Hyperlipidemia, fatty liver, and bromsulfophthalein  
retention in rabbits injected intravenously with bacterial endoxins.  
J. Lipid Res. 5: 563-568.
48. Mukherjee, K. L., A. K., Bhattacharyya, R. M. Smith, T. M. Mueller  
48. Mukherjee, K. L., A. K. Bhattacharyya, R. M. Smith, T. M. Mueller,  
and T. M. Smith. 1973. Lipid metabolism in septic shock.  
Changes in the lipid metabolism of mice with lethal Staphylococcus  
aureus septic shock. Can. J. Surg. 16: 45-51.
49. Canonico, P. G., E. Ayala, W. L. Rill, and J. S. Little. 1977.  
Effects of pneumococcal infection on rat liver microsomal enzymes  
and lipogenesis by isolated hepatocytes. Am. J. Clin. Nutr.  
30: 1359-1363.
50. McGarry, J. D., G. P. Mannaerts, and D. W. Foster. 1977. A  
possible role for malonyl-CoA in the regulation of hepatic fatty  
acid oxidation and ketogenesis. J. Clin. Invest. 60: 265-270.
51. Cook, G. A., M. T. King, and R. L. Veech. 1978. Ketogenesis  
and malonyl coenzyme A content of isolated rat hepatocytes.  
J. Biol. Chem. 253: 2529-2531.

TABLE I

In Vitro Rates of Lipolysis and Esterification in Epididymal Fat Pads of Fed or Fasted Rats Exposed to Liver or Heat-killed S. pneumoniae

Treatment	Number of pairs	Weight g	Glycerol production $\mu\text{mol/g/h}$	Free fatty acid production $\mu\text{eq/g/h}$	Free fatty acid esterification $\mu\text{eq/g/h}$	%
Fed	15	$2.95 \pm 0.21$	$2.21 \pm 0.26$	$0.29 \pm 0.02$	$6.07 \pm 0.77$	$95.3 \pm 0.4$
Fasted						
Heat-killed						
24 h	15	$2.80 \pm 0.24$	$3.28 \pm 0.15^*$	$1.69 \pm 0.22^*$	$8.46 \pm 0.39^*$	$81.8 \pm 1.9^*$
48 h	10	$1.79 \pm 0.19^*$	$3.82 \pm 0.12^*$	$2.79 \pm 0.27^{*\dagger}$	$8.67 \pm 0.41^*$	$75.5 \pm 2.4^*$
Infected						
24 h	9	$2.68 \pm 0.25$	$3.09 \pm 0.15^*$	$2.18 \pm 0.23^*$	$7.10 \pm 0.41$	$76.4 \pm 2.4^*$
48 h	11	$1.95 \pm 0.20^*$	$3.24 \pm 0.16^*$	$2.19 \pm 0.20^*$	$7.54 \pm 0.51$	$77.5 \pm 2.5^*$

Data are presented in mean  $\pm$  SE.

\*  $P < 0.01$  vs. fed.

$\dagger P < 0.01$  vs. 24-h, fasted, heat-killed control.

TABLE II

Effect of Fasting and S. Pneumoniae Infection on In VivoRate of Oxidation of Prelabeled Body Fat

Treatment	Rate of [ $^{14}\text{CO}_2$ ] production	Percent of total body [ $^{14}\text{C}$ ] loss per day
	<u>dpm/day <math>\times 10^{-5}</math></u>	<u>%/day</u>
Fed	1.75 $\pm$ 0.11	6.68 $\pm$ 0.39
Fasted		
Heat-killed		
24 h	2.42 $\pm$ 0.29 <sup>*</sup>	9.26 $\pm$ 1.11
48 h	3.28 $\pm$ 0.20 <sup>*</sup>	14.94 $\pm$ 1.35 <sup>*†</sup>
Infected		
24 h	2.28 $\pm$ 0.13	9.50 $\pm$ 0.96
48 h	2.76 $\pm$ 0.26 <sup>*</sup>	13.03 $\pm$ 1.67 <sup>*</sup>

Data are mean  $\pm$  SE of 6 rats.

<sup>\*</sup> $\underline{P} \leq 0.01$  vs. fed.

<sup>†</sup> $\underline{P} \leq 0.01$  vs. 24-h, fasted, heat-killed controls.



TABLE III

In Vivo Rates of [ $^{14}\text{C}$ ] production from 1- $^{14}\text{C}$ Palmitic or  
1- $^{14}\text{C}$ Oleic Acid by Fasted Rats Exposed to Heat-killed or Live  
S. Pneumoniae

Treatment	Oleic acid	Palmitic acid
	<u>% of injected dose</u> <u>exhaled as [<math>^{14}\text{C}</math>]</u>	
Heat-killed		
24 h	53.5 $\pm$ 5.2	39.6 $\pm$ 2.1 <sup>*†</sup>
48 h	36.7 $\pm$ 3.5	33.3 $\pm$ 2.6 <sup>*†</sup>
Infected		
24 h	55.1 $\pm$ 4.9	38.7 $\pm$ 3.1 <sup>*†</sup>
48 h	44.7 $\pm$ 1.6	38.4 $\pm$ 2.7 <sup>*†</sup>

Rats injected i.v. with 1  $\mu\text{Ci}$ /100 g body wt of labeled fatty acid.

Data are mean  $\pm$  SE of 6 rats.

\*  $\underline{p} \leq 0.01$  vs. 24-h, oleic acid, heat-killed.

†  $\underline{p} \leq 0.01$  vs. 24-h, oleic acid infected.

TABLE IV

Effect of S. Pneumoniae Infection on Plasma  
Albumin and Free Fatty Acid Concentrations

Treatment	Albumin	Free fatty acid	Fatty Acid albumin
	<u>g/dl</u>	<u>μeq/liter</u>	<u>molar ratio</u>
Heat-killed			
24 h	2.40 ± 0.07	740 ± 44	2.07 ± 0.12
48 h	2.22 ± 0.06	622 ± 31	1.88 ± 0.09
Infected			
24 h	2.09 ± 0.07	581 ± 41	2.13 ± 0.21
48 h	0.93 ± 0.11 <sup>*</sup>	140 ± 16 <sup>*</sup>	2.33 ± 0.27

Data are mean ± SE of 8 rats.

\*  $P \leq 0.01$  vs. 48-h heat-killed.

TABLE V

Rate of Ketone Production From Oleic or Octanoic Acid by Perfused 35  
Livers from Fed or Fasted Rats Exposed to Live or Heat-killed (HK)  
*S. pneumoniae* or *F. tularensis* (LVS)

Treatment	Ketogenesis		
	KRB + Alb	KRB + alb + 20 mM oleic acid	KRB + alb + 20 mM octanoic acid
	$\mu\text{mol/min/100 g body wt (n)}$		
Fed	$0.43 \pm 0.07$ (6)*	$0.75 \pm 0.09$ (10)	$1.02 \pm 0.21$ (6)
Fasted			
HK <i>S. pneumoniae</i>			
24 h	-	$2.17 \pm 0.22^*$ (5)	$1.23 \pm 0.09$ (5)
48 h	$0.53 \pm 0.27$ (5)	$2.10 \pm 0.11^*$ (11)	$1.67 \pm 0.08^*$ (17)
Live <i>S. pneumoniae</i>			
24 h	-	$1.89 \pm 0.11^*$ (5)	$1.24 \pm 0.19$ (3)
48 h	$0.58 \pm 0.05$ (5)	$1.21 \pm 0.09^{*\dagger\ddagger  }$ (10)	$1.77 \pm 0.09^*$ (13)
HK LVS			
24 h	-	$1.98 \pm 0.20^*$ (7)	$1.58 \pm 0.11^*$ (7)
48 h	-	$1.69 \pm 0.07^*$ (7)	$1.70 \pm 0.20^*$ (6)
Live LVS			
24 h	-	$1.52 \pm 0.21^{*\dagger\ddagger}$ (7)	$1.46 \pm 0.17$ (7)
48 h	-	$1.08 \pm 0.11^{\dagger\ddagger***\dagger\dagger}$ (7)	$1.39 \pm 0.06$ (6)

Data are mean  $\pm$  SE.

\*  $P < 0.01$  vs. fed.

$\dagger P < 0.01$  vs. 24-h, HK *S. pneumoniae*.

$\ddagger P < 0.01$  vs. 48-h, HK *S. pneumoniae*.

$|| P < 0.01$  vs. 24-h, live *S. pneumoniae*.

\*\*  $P < 0.01$  vs. 24-h, HK LVS.

$\dagger\dagger P < 0.01$  vs. 48-h, HK LVS.



TABLE VI

Rate of Oleic Acid Uptake by Perfused Livers from Fasted Rats 48 h after Inoculation with Live or Heat-killed *S. pneumoniae* or *F. tularensis* (LVS)

Treatment	<u><i>S. pneumoniae</i></u>	LVS
	<u><math>\mu\text{mol/min/100 g body wt}</math></u>	
Heat-killed	$0.74 \pm 0.02$	$0.74 \pm 0.03$
Infected	$0.70 \pm 0.05$	$0.60 \pm 0.03^*$

Data are mean  $\pm$  SE of 6 rats.

\*  $P \leq 0.01$  vs. heat-killed.

TABLE VII

Metabolism of 1-[<sup>14</sup>C]Oleic Acid by Perfused Livers from Fasted Rats 48 h after  
Inoculation with Heat-killed or Live *S. pneumoniae*

Treatment	Oleic acid used	Ketones	CO <sub>2</sub>	Liver lipids
<u>μmol/100 g liver</u>				
Heat-killed	38.2 ± 0.5	26.7 ± 1.5	1.4 ± 0.2	8.5 ± 0.6
Infected	37.2 ± 1.4	13.3 ± 1.9	1.1 ± 0.1	21.8 ± 0.5*

Data are ± SE of 6 livers.

\* P ≤ 0.01 vs. heat-killed.

TABLE VIII

Effect of Fasting and *S. pneumoniae* Infection on Liver Weight, Lipid Content and Incorporation of [ $^{14}\text{C}$ ]Octanoic or Oleic Acid into Liver Lipid during *in vitro* Perfusion Studies

Treatment	Liver		Liver lipid	
	wt	Weight	[ $^{14}\text{C}$ ]Octanoic	[ $^{14}\text{C}$ ]Oleic
	g	mg/liver	dpm/total liver lipid $\times 10^6$	
Fed	8.16 $\pm$ 0.21	552 $\pm$ 29	1.33 $\pm$ 0.17	5.84 $\pm$ 0.21
Fasted				
Heat-killed				
24 h	6.52 $\pm$ 0.11	326 $\pm$ 26*	0.35 $\pm$ 0.02*	2.71 $\pm$ 0.20*
48 h	6.20 $\pm$ 0.19*	300 $\pm$ 13*	0.24 $\pm$ 0.03*	2.34 $\pm$ 0.17*
Infected				
24 h	7.13 $\pm$ 0.20*§	374 $\pm$ 20*	0.68 $\pm$ 0.07*§	3.62 $\pm$ 0.17*§
48 h	7.89 $\pm$ 0.15	464 $\pm$ 21	1.30 $\pm$ 0.12  ¶	5.27 $\pm$ 0.14  ¶

Data are  $\pm$  SE of 6 rats.

\*  $\underline{p} < 0.01$  vs. fed.

§  $\underline{p} < 0.01$  vs. 24-h, fasted heat-killed.

||  $\underline{p} < 0.01$  vs. 48-h, fasted heat-killed.

¶  $\underline{p} < 0.01$  vs. 24-h, infected.



TABLE IX

In vivo Rate of [ $^{14}\text{C}$ ] Production and Oxidization of  $\beta$ -3-[ $^{14}\text{C}$ ]Hydroxybutyrate or 3-[ $^{14}\text{C}$ ]Acetoacetate by Fasted Rats Exposed to Heat-killed or Live *S. pneumoniae*

Treatment	$\beta$ -hydroxy- butyrate	Acetoacetate	$\beta$ -hydroxy- butyrate	Acetoacetate	$\beta$ -hydroxy- butyrate	Acetoacetate	$\mu\text{mol oxidized/min/100 g body wt}$
<u>Fasted</u>							
<u>Heat-killed</u>							
24 h	2.40 $\pm$ 0.38	2.43 $\pm$ 0.48	100.9 $\pm$ 4.8	9.42 $\pm$ 0.39	2.39 $\pm$ 0.14	0.24 $\pm$ 0.03	
40 h	3.47 $\pm$ 0.38	-	89.2 $\pm$ 5.1	-	3.10 $\pm$ 0.43	-	
48 h	1.96 $\pm$ 0.27	-	121.7 $\pm$ 15.9	-	3.10 $\pm$ 0.68	-	
<u>Infected</u>							
24 h	2.43 $\pm$ 0.33	2.00 $\pm$ 0.15	59.1 $\pm$ 7.0	6.73 $\pm$ 0.57 <sup>†</sup>	1.49 $\pm$ 0.18	0.13 $\pm$ 0.01	
40 h	3.68 $\pm$ 0.83	-	31.7 $\pm$ 4.5 <sup>†</sup>	-	0.94 $\pm$ 0.39	-	
48 h	0.73 $\pm$ 0.23 <sup>†</sup>	-	13.7 $\pm$ 4.5 <sup>†</sup>	-	0.12 $\pm$ 0.06 <sup>†</sup>	-	

Data are mean  $\pm$  SE of 6 rats.

\* Percentage of a 1  $\mu\text{Ci}/100$  g body wt dose given i.v.

<sup>†</sup>  $p < 0.01$  vs. fasted, heat-killed control.

Legend to figure

FIGURE 1 Effects of a pneumococcal infection and the disposal and utilization of an i.v. lipid load. Both infected (■) and control (●) rats were injected i.v. with 20 mg/100 g body wt of a lipid solution. At 0, 15, 30, 60, 120, 180 and 240 min after the lipid load, plasma was analyzed for triglycerides, fatty acids,  $\beta$ -hydroxybutyrate, cholesterol, [ $^{14}$ C]tripalmitin and glucose by procedures described in Methods. The rats to be killed at 240 min were injected with 20 mg of lipid plus 1  $\mu$ Ci of [ $^{14}$ C]tripalmitin/100 g body wt and utilized to measure [ $^{14}$ CO $_2$ ] production as described in Methods. Values are mean of 6 rats. Points with vertical lines indicate mean  $\pm$  SE of values with significant differences ( $P < 0.01$ ) between infected and control rats.

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